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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/435, A61K 39/00	A1	(11) International Publication Number: WO 99/58564 (43) International Publication Date: 18 November 1999 (18.11.99)
(21) International Application Number: PCT/NO99/00141 (22) International Filing Date: 30 April 1999 (30.04.99) (30) Priority Data: 19982098 8 May 1998 (08.05.98) NO (71) Applicant (for all designated States except US): NORSK HYDRO ASA [NO/NO]; N-0240 Oslo (NO). (72) Inventors; and (75) Inventors/Applicants (for US only): GAUDERNACK, Gustav [NO/NO]; Anthon Walles vei 17 A, N-1300 Sandvika (NO). ERIKSEN, Jon, Amund [NO/NO]; Bjørntvedt gt. 37, N-3916 Porsgrunn (NO). MØLLER, Mona [NO/NO]; Skrukkerødtoppen 8, N-3925 Porsgrunn (NO). (74) Agent: LILLEGRAVEN, Rita; Norsk Hydro ASA, N-0240 Oslo (NO).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: FRAMESHIFT MUTANTS OF BETA-AMYLOID PRECURSOR PROTEIN AND UBIQUITIN-B AND THEIR USE		
(57) Abstract Frameshift Mutants β -Amyloid precursor peptides and mutant ubiquitin-B associated with Alzheimer's disease and Down syndrome eliciting T cellular immunity for use in compositions for the treatment and/or prophylaxis of Alzheimer's disease and/or Down syndrome.		

FRAMESHIFT MUTANTS OF BETA-AMYLOID PRECURSOR PROTEIN AND
UBIQUITIN-B AND THEIR USE

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5 The present invention relates to peptides for treatment
and/or prophylaxis of Alzheimer's disease and Down
syndrome.

10 Alzheimer's disease and treatment of Down syndrome are both
associated with frameshift mutations occurring at the
transcriptional level or by posttranscriptional editing of
RNA during the encoding of β -Amyloid precursor protein
(β APP) and ubiquitin-B (Ubi-B). Such frameshift mutations
15 give rise to mutant β APP and Ubi-B protein products which
are characterised by aberrant protein sequences at the
carboxyl terminus. Peptides covering, either completely or
parts of, the aberrant parts of mutant β APP or Ubi-B
protein products elicit T cellular immunity and can
therefore be useful in compositions for the treatment of
20 Alzheimer's disease and Down syndrome. Further the peptides
of this invention can be used as a prophylactic
anti-Alzheimer's disease vaccine.

25 The invention also relates to DNA sequences encoding
peptides corresponding to aberrant β APP and Ubi-B protein
sequences found in Alzheimer's disease and Down syndrome
patients, and to vectors comprising at least one insertion
site containing a DNA sequence encoding at least one such
peptide.

30 Further the invention relates to methods for the treatment
and/or prophylaxis of Alzheimer's disease by administration
of at least one mutant β APP and/or Ubi-B peptide or a
recombinant virus vector comprising at least one insertion

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In Alzheimer's and Down syndrome patients, intracellular and extracellular deposits of proteins in tangles, neurophil threads and neuritic plaques are correlated with neuronal dysfunction leading to dementia (R.D.Terry et al in *Alzheimer Disease*, R.D.Terry, R.Katzman, K.L.Bick, Eds. (Raven, New York, 1994) pp. 179-196). These protein deposits have been shown to contain forms of β amyloid precursor protein (β APP) and ubiquitin-B (Ubi-B) that are aberrant in the carboxyl terminus, and it has further been shown that these aberrant protein sequences are results of frameshift mutations which probably occur at the transcriptional level or by posttranscriptional editing of RNA (F.W. van Leeuwen et al, *Science*, vol 279, pp. 242-247).

In the case of β APP two frameshift mutations have been observed, one by deletion of the di-nucleoside deoxyguanosine-deoxyadenosine (GA) unit from the (ACC)GAGAGAGA(ATG)_^ sequence in exon 9, and one by deletion of a GA unit from the (CAT)GAGAGA(ATG)_^ sequence in exon 10. The mutant β APP peptides resulting from these frameshift mutations are shown in table 1. The peptides with seq id nos 1 and 4 are the mutant part of the β APP protein sequence and the peptides with seq id nos 2, 3 and 5 represent mutant peptides extended into the normal β APP sequence at the amino terminus.

normal β APP; RLEAKHRERMSQVMREWEEAERQAKNLPK (SEQ ID NO:13)
 seq id no 1; NVPGHERMGRGRTSSKELA
 seq id no 2; RLEAKHRENVPGHERMGRGRTSSKELA
 seq id no 3; RLEAKHRENVPGHERMG
 seq id no 4; MGRGRTSSKELA
 seq id no 5; ERMSQVMRMGRGRTS

Table 1.

Also in the case of Ubi-B two frameshift mutations have been observed, one by deletion of the di-nucleoside deoxyguanosine-deoxythymidine (GT) unit from the (TCT)GAGAGGT(GGT)^{seq ID NO: 14} sequence in exon, and one by deletion of a di-nucleoside deoxycytosine-deoxythymidine (CT) unit from the (TCA)CTCT(GGA)^{seq ID NO: 15} sequence in exon. The mutant Ubi-B peptides resulting from these frameshift mutations are shown in table 2. The peptides with seq id nos 6 and 9 are the mutant part of the Ubi-B protein sequence and the peptides with seq id nos 7, 8 and 10 represent mutant peptides extended into the normal Ubi-B sequence at the amino terminus.

normal Ubi-B;	HLVLRLRGGMQIFVKTLTGKTITLEVEPSD (seq ID NO: 16)
seq id no 6;	YADLREDPDRQDHHPGSGAQ
seq id no 7;	HLVLRLRGYADLREDPDRQDHHPGSGAQ
seq id no 8;	HLVLRLRGYADLREDPD
seq id no 9;	GGGAQ
seq id no 10;	TLTGKTITGGGAQ

Table 2.

The mutant β APP and Ubi-B proteins are only encoded for by cells in which corresponding frameshift mutations have occurred and are therefore targets for specific immunotherapy of Alzheimer's disease and Down syndrome.

According to the present invention, peptides corresponding to mutant β APP and mutant Ubi-B proteins can be used to elicit T cellular immunity and specific killing of cells producing mutant β APP and mutant Ubi-B proteins, which in Alzheimer's disease and Down syndrome patients are correlated with neuronal dysfunction leading to dementia.

of immunostimulatory DNA sequences (ISS). These can take the form of hexameric motifs containing methylated CpG, according to the formula :

5'-purine-purine-CG-pyrimidine-pyrimidine-3'. Our DNA vaccines may therefore incorporate these or other ISS, in the DNA encoding the peptides, in the DNA encoding the cytokine or other co-stimulatory molecules, or in both. A review of the advantages of DNA vaccination is provided by Tighe et al (1998, *Immunology Today*, 19(2), 89-97).

In one embodiment, the DNA sequence encoding the mutant β APP and mutant Ubi-B peptides comprises:

Normal β APP gene sequence (exons 9 and 10).

repeat 1

GAG AGG CTT GAG GCC AAG CAC CGA GAG AGA ATG TCC CAG GTC ATG (SEQ ID NO:17)

repeat 2

AGA GAA TGG GAA GAG GCA GAA CGT CAA GCA AAG AAC TTG CCT AAA (SEQ ID NO:18)

Mutant β APP gene sequence, GA deleted from repeat 1.

GAG AGG CTT GAG GCC AAG CAC CGA GAG AAT GTC CCA GGT CAT GAG (SEQ ID NO:19)
AGA ATG GGA AGA GGC AGA ACG TCA AGC AAA GAA CTT GCC TAA

Mutant β APP gene sequence, GA deleted from repeat 2.

GAG AGG CTT GAG GCC AAG CAC CGA GAG AGA ATG TCC CAG GTC ATG
AGA ATG GGA AGA GGC AGA ACG TCA AGC AAA GAA CTT GCC TAA (SEQ ID NO:20)

Normal Ubi-B gene (exon) sequence.

deletion motif

CAC CTG GTC CTG CGT CTG AGA GGT GGT ATG CAG ATC TTC GTG AAG
ACC CTG ACC GGC AAG ACC ATC ACC CTG GAA GTG GAG CCC AGT GAC (SEQ ID NO:21)

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Mutant Ubi-B gene sequence, GT deleted from the deletion motif.

CAC CTG GTC CTG CGT CTG AGA GGG TAT GCA GAT CTT CGT GAA GAC
CCT GAC CGG CAA GAC CAT CAC CCT GGA AGT GGA GCC CAG TGA (SEQ ID NO: 22)

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Normal Ubi-B gene (exon 2) sequence.

CAC CTG GTC CTG CGT CTG AGA GGT GGT ATG CAG ATC TTC GTG AAG (SEQ ID NO: 23)
CT repeat

ACC CTG ACC GGC AAG ACC ATC ACT CTG GAG GTG GAG CCC AGT GAC (SEQ ID NO: 24)

Mutant Ubi-B gene sequence, CT deleted from the CT repeat.

CAC CTG GTC CTG CGT CTG AGA GGT GGT ATG CAG ATC TTC GTG AAG
ACC CTG ACC GGC AAG ACC ATC ACT GGA GGT GGA GCC CAG TGA (SEQ ID NO: 25)

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The invention further encompasses vectors and plasmids comprising a DNA sequence encoding at least one frameshift mutant β APP and/or Ubi-B peptide. The vectors include, but are not limited to *E. Coli* plasmid, a *Listeria* vector and recombinant viral vectors. Recombinant viral vectors include, but are not limited to orthopox virus, canary virus, capripox virus, suipox virus, vaccinia, baculovirus, human adenovirus, SV40, bovine papilloma virus and the like comprising the DNA sequence encoding a mutant β APP and/or Ubi-B peptide.

It is considered that a treatment for Alzheimer's disease and Down syndrome, or prophylaxis for Alzheimer's disease, may be achieved also through the administration of an effective amount of a recombinant virus vector or plasmid comprising at least one insertion site containing a DNA sequence encoding a frameshift mutant peptide to a patient.